CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

TRIBUTYLPHOSPHOROTRITHIOATE
(DEF)
Chemical Code # 190, Tolerance # 00272
SB 950 # 152

November 19, 1987
Revised: 2/27/89, 8/2/89, 8/06/90, 7/1/91, 1/24/92, 7/30/92, 12/21/92, 9/20/00, 6/11/01, 10/5/01, 5/15/02

I. DATA GAP STATUS

Chronic, rat:	No data gap, possible adverse effect.
Chronic toxicity, dog:	No data gap, possible adverse effect.
Oncogenicity, rat:	No data gap, no adverse effect.
Oncogenicity, mouse:	No data gap, possible adverse effect.
Reproduction, rat:	No data gap, possible adverse effect.
Teratology, rat:	No data gap, no adverse effect.
Teratology, rabbit:	No data gap, no adverse effect.
Gene mutation:	No data gap, no adverse effect.
Chromosome effects:	No data gap, no adverse effect.
DNA damage:	No data gap, no adverse effect.
Neurotoxicity:	No data gap, possible adverse effect.[a]

Toxicology one-liners are attached.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T020515
All record numbers through 182690 (Document No. 272-114) were examined, plus all record numbers > 900000.
Revised by Gee, 2/27/89, and 8/2/89. Revised by Aldous, 8/06/90. Revised by Kellner 7/1/91, 1/24/92, 7/30/92 and 12/21/92. Revised by Gee, 9/20/00, 6/11/01, 10/5/01 and 5/15/02.

These pages contain summaries only. Each individual worksheet may contain additional effects.

[a] There are several studies in the rat including a subchronic study showing retinal atrophy.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC TOXICITY, RAT

005 919138, "Chronic Oral Toxicity of DEF to Male and Female Rats" (Univ. of Chicago (4/18/67)) DEF (97.7%) was fed in the diet to groups of 24/sex/group at 0, 5 25, 100, or 250 ppm for 2 years. **Possible adverse effect**: NOEL = 25 ppm for liver cytoplasmic vacuolation in females. Cholinesterase inhibition = 5 ppm (plasma and RBC ChE inhibition at 25 ppm). UNACCEPTABLE, NOT UPGRADEABLE due to histopathological examinations of only 5/sex/group, major organs only, no hematology, no clinical chemistry, no analysis of dose, pneumonia in almost all animals. Summary only. (A. Apostolou 5/30/85).

EPA one-liner: No core grade. ChE NOEL < 5 ppm (LTD) (possible ChE inhibition in female RBC); Systemic NOEL = 25 ppm (decreased body weights, cytoplasmic vacuoles in liver cells (fatty changes), determination of validity pending submission of table 5 judged illegible and correction of 2 typographical errors.)

CHRONIC TOXICITY, DOG

036 069777 Protocol for chronic study in 272-050

** **272-050 96421** Christenson, W. "Chronic Feeding Toxicity Study of Technical Grade Tribufos (DEF®) with Dogs" (Mobay Report No. 100653, Mobay Corporation, Study No. 88-274-AB, Feb. 26, 1991.) Tribufos technical, purity 98.7%, batch # 85R26-39, at nominal concentrations of 0 (control), 4, 16 and 64 ppm in feed was administered to 4 Beagle dogs/sex/dietary level for one year. Observations for body weights, food consumption, clinical signs, palpable masses and ophthalmologic lesions showed no compound-related effects. Plasma cholinesterase (ChE) was significantly depressed at the 4, 16 and 64 ppm dose levels in males and at the 16 and 64 ppm levels in females. Brain ChE was not inhibited one week after dosing ceased. Male cholinergic NOEL < 4 ppm (0.1 mg/kg/day); female cholinergic NOEL = 4 ppm (0.1 mg/kg/day). **Possible Adverse Effect**: Females had symptoms of anemia (lowered RBC counts, hemoglobin and % hematocrit). Female anemia NOEL = 16 ppm (0.4 mg/kg/day); male anemia NOEL = 64 ppm (1.6 mg/kg/day). Acceptable (Kellner and Gee, 6/24/91).

COMBINED CHRONIC/ONCO, RAT

036 069771, Protocol (including amendments) for combined study -072 114647.

038 071399, Protocol for combined study -072 114647; also includes 5 tables which summarize the results of subacute and chronic oral toxicity studies performed in rats which were used to justify the dose levels used in the combined rat study. Kellner, 7/30/92.

** **272-072 114647** Christenson, W. "Technical Grade Tribufos (DEF®): A Chronic Toxicity/Oncogenicity/Neurotoxicity Feeding Study in the Fischer 344 Rat" (Miles Inc., Report # 102675, May 1, 1992). Tribufos technical, purity 98.5%, batch # 85R26-39, at nominal concentrations of 0(control), 4, 40 and 320 ppm in feed was administered to a one-year interim sacrifice/chronic toxicity group (20 rats/sex/dose in control and 320 ppm, 10/sex/dose in remaining groups), a two-year oncogenicity group (50/sex/dose) and a one- and two-year

neurotoxicity group (10/sex/dose for each year). Average monthly body weight gain for 320 ppm-dose oncogenicity rats was 73% of control after two years. Increase in diffuse, bilateral retinal atrophy in one- and two-year 320 ppm rats and increased optic nerve atrophy, cataract, corneal opacity, corneal neovascularization, iritis/uveitis, lens opacity and unrecordable (flat) bilateral electroretinographic responses were reported in two-year 320 ppm rats. Indications of anemia and declines in total protein, globulin, cholesterol, calcium, ALP, ALT were seen at 40 ppm and above. Hyperplasia and vacuolation of the mucosa of the proximal small intestine was seen in one- and two-year rats down to 40 ppm. Increased BUN and vacuolation of the adrenal cortex was seen in 320 ppm two-year rats. Increased testicular weight in two-year males and increased adrenal weight in one- and two-year rats was reported at 320 ppm. No Adverse Effects for oncogenicity or neurotoxicity. **Possible Adverse Effects (Chronic toxicity): Hyperplasia of small intestine, bilateral retinal atrophy (and related secondary ocular effects) and clinical biochemical changes; NOEL = 4 ppm (0.2 mg/kg/day).** ACCEPTABLE. Kellner and Gee, 7/17/92.

272-085 119087 [Addendum to -072:114647] Supplemental submission (Miles Report # 102675-1) provided additional ophthalmic and electroretinographic (ERG) data that were requested by DPR. Specifically, numbers of animals submitted from each group for ophthalmologic examination (to obtain percentage of animals with ocular lesion) and missing summary Tables OP5K-SUM and OP6K-SUM (ERG data) were requested. This information did not change the conclusions presented in the original study. Kellner and Gee, 12/16/92.

ONCOGENICITY, MOUSE

** **272-041 076144** Hayes, R. H., "Oncogenicity study of technical grade Tribufos (DEF®) with mice" (Mobay Report No. 99175). Mobay Corporation, Study no. 86-271-01, June 29, 1989. Tribufos DEF® technical, purity 98.6% at nominal concentrations of 0 (Mazola oil), 10, 50 or 250 ppm to 50 CD-1 mice/sex/group for 90 weeks. There was evidence of anemia: Hematology NOEL = 50 ppm (both sexes). Possibly related extramedullary hematopoiesis in spleen was elevated in 50 ppm and 250 ppm males. Cholinesterase (ChE) NOEL for males/females < 10 ppm [marked plasma ChE inhibition, and modest (statistically significant, and possibly biologically significant) RBC ChE inhibition at 10 ppm]. Histopathology NOEL = 10 ppm [primarily due to vacuolar degeneration of small intestine (M, F)]. **Possible adverse effects**, all noted at 250 ppm, included adenocarcinomas of small intestine (attributed to treatment in both sexes, but statistically significant only in males); liver hemangiosarcomas (males); and lung alveolar/bronchiolar adenomas (females). Evidences of 250 ppm being over the MTD include increased mortality (both sexes); consistent depression of hematological indices, suggesting anemia (both sexes); degeneration of adrenal cortex and of proximal segments of the small intestine (both sexes); and dilatation or edema of the cecum (both sexes). Indications of high dose toxicity should be considered in risk assessment, but do not remove the "possible adverse effects" flag. Acceptable. (Kishiyama/C. Aldous, 8/6/90).

020 038470 pilot study for 041:076144, above.

026 058613 Interim report for 041:076144, above.

037 A letter dated February 7, 1989 indicating a possible adverse effect in the mouse study above (tumors in small intestine) in study 041:076144, above.

REPRODUCTION, RAT

036 069776, Protocol for reproduction study -063 98635.

** **272-063 98635** Eigenberg, D. "A Two-Generation Dietary Reproduction Study in Rats Using Tribufos (DEF®)", (Mobay Corp., Health, Environment, Safety and Plant Management, Corporate Toxicology Dept., Report No. 101255, 9/10/91). Tribufos technical, lot 85R-26-39, 98.5% purity, was administered in the diet to Sprague-Dawley (CD) rats for 2 generations at nominal concentrations of 0 (control), 4, 32 and 260 ppm beginning ten weeks prior to mating with 30 rats/sex/group. Lower body weight gain during all phases and lower food consumption during lactation was seen in F0 and F1 adults. Depression of brain, plasma and erythrocyte cholinesterase (ChE) was seen in adults and F1a and F2a pups: parental ChE NOEL = 4 ppm; Pup ChE NOEL = 32 ppm. **Possible adverse effects**: Cannibalization of pups; increased gestation length and litters with stillborn pups and decreased litter weight. Reduction in the following indices: fertility, birth, live birth and viability; Reproductive NOEL = 32 ppm (2.06 mg/kg/day for females during gestation). Originally unacceptable because the description of dosing scheme and summarization of data was inadequate; reporting of live litters was inconsistent. Upgraded to ACCEPTABLE status upon review of revised tables in supplementary submission -075:114893 from Miles, Inc. Kellner and Gee, 8/4/92.

272-075 114893 [Addendum to -063:98635] This supplemental submission provided clarification for the method used to calculate the following indices: live birth index, viability index and birth index; In addition, corrections where made to various tables (entire report was re-checked for errors by the sponsor and the modified pages were presented in numerical order for replacement in the original report); no changes in the study conclusions were made. These corrections permit an upgrade of study 063:98635 to acceptable status. Supplemental Data. Kellner and Gee, 8/4/92.

272-062 98674 Eigenberg, D. "A Cross-Fostering Study in Rats Using Tribufos (DEF®) Administered in the Diet" (Mobay Corp., Health, Environment, Safety and Plant Management, Corporate Toxicology Dept., Report No. 101254, 8/29/91). Tribufos technical, lot 85R-26-39, 98.5% purity was administered in the diet of 60 male and 130 female Sprague-Dawley (CD) rats divided into four groups of 15 males and 30 females/group at nominal doses of 0 (control) and 260 ppm (2 groups each) for ten weeks. Pups from groups 1 (0 ppm) and 3 (260 ppm) were cross-fostered between dose groups and pups from groups 2 (0 ppm) and 4 (260 ppm) were cross-fostered to dams within the group. Cholinergic symptoms: Muscle fasciculations in 11.7% of 260 ppm-dosed dams. **Possible Adverse Effects**: Cannibalization of pups and reduction of pup body weight gain was due to a compound effect on the dam (e.g. it occurred primarily in dose groups in which pups were nurtured by dams which received 260 ppm tribufos); reduction in pup viability was the result of a combined effect on the dam and the pup. Supplement to study 98635. (Kellner and Gee, 1/13/92).

272-062 98634 Eigenberg, D. "A Dietary Reproductive Toxicity Study Investigating the Fertility of F1 Rats Using Tribufos (DEF®)" (Mobay Corp., Health, Environment, Safety and Plant Management, Corporate Toxicology Dept., Report No. 101256, 8/27/91). Tribufos technical, lot 85R-26-39, 98.5% purity, was administered in the diet of 60 male and 60 female Sprague-Dawley rats divided into 2 groups of 30 rats/sex/group at 0 (control) and 260 ppm for 10 weeks prior to mating. Females were sacrificed on day 20 of gestation to confirm pregnancy. The results were equivocal, with a reduction in the fertility index seen (93% versus 83% in the control and high-dose groups, respectively) which was not statistically significant; this was similar to the outcome obtained in the two-generation reproduction study. This study could not be used to state

definitively what effect tribufos (260 ppm) had on fertility. Supplement to study 98635. (Kellner and Gee, 1/13/92).

TERATOLOGY, RAT

** 024 050639, "A Teratology Study with DEF Technical in the Rat" (Miles Labs, Mobay No. 87320, 8-8-86) DEF Technical 98% pure; 0, 1, 7, or 28 mg/kg to 33/group on Day 6-15 of gestation; statistically significant reduction of plasma and erythrocyte ChE at mid and high dose, maternal brain ChE activity at high dose; no teratology effects; Maternal NOEL = 1 mg/kg, Developmental NOEL = 28 mg/kg. No adverse effect indicated. ACCEPTABLE. (J. Parker, 3/11/87).

TERATOLOGY, RABBIT

** 027 058615, "Teratology Study in the Rabbit with DEF Technical", (Miles Laboratories, Report No. MTD0003, 12/22/87, Mobay report 94468). DEF, purity 98%, administered by gavage at doses of 0 (CMC vehicle), 1, 3, or 9 mg/kg on day 7 through 19 of gestation to 17 American Dutch rabbits/group. Systemic maternal NOEL = 3 mg/kg/day (reduced bodyweight gain); Maternal ChE < 1 mg/kg/day, plasma (on day 20) and erythrocyte (on day 20 and 28) inhibition for DEF treated groups. Developmental NOEL = > 9 mg/kg/day. No adverse developmental effects. ACCEPTABLE. (JSK, 12/5/88 and Gee, 2/27/89.)

272-030 Exact duplicate of 027:058615, above.

GENE MUTATION

** 040 074445 "Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay," (Microbiological Associates, Study No. T8299.501, Mobay No. 98575) DEF technical, 98.5%, was tested with Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 at 0 (DMSO), 667, 1000, 3333, 6667 and 10,000 ug/plate in the plate incorporation method. All concentrations were plated in triplicate, no repeat trial. There was a slight precipitate at 667 ug/plate and above without activation and at 1000 ug/plate and above with activation. No adverse effect, Acceptable. D. Shimer, 7/21/89, J. Gee, 8/2/89.

CHROMOSOME EFFECTS

** 039 074161 "Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells" (Microbiological Associates, Inc., Study No. T8299.337, Mobay No. 98592) DEF Technical, 98.5% purity, was tested in the chromosome aberration assay using Chinese hamster ovary cells. With metabolic activation dose levels of 0 (DMSO), 0.007, 0.013, 0.25, 0.050 and 0.10 ul/ml were used. In the absence of activation dose levels of 0 (DMSO), 0.004, 0.007, 0.013, 0.025 and 0.050 ul/ml were used. The two highest concentrations in each treatment had reduced mitotic index and cell toxicity observed by microscopic examination. Cells were collected after 20 hours. Duplicate cultures were used with a total of 100 cells being counted for each dose level. No adverse effect. Acceptable. D. Shimer, 7/21/89, J. Gee, 8/2/89.

DNA DAMAGE

** 040 074444 "Unscheduled DNA Synthesis in Rat Primary Hepatocytes" (Microbiological Associates, Lab No. T8299.380, Mobay No. 98576, 3-3-89) Primary rat hepatocytes (uninduced) were treated with DEF technical, 98.5%, for 18 to 20 hours at concentrations of 0(DMSO), 0.0001, 0.0003, 0.001, 0.003, 0.006, 0.01 and 0.03 ul/ml, concentrations selected after a preliminary cytotoxicity test. Concentrations of 0.01 and 0.03 were too toxic to be evaluated. Relative survival of the remaining cultures was 84% to >100%. Glass plates were used in the final assay rather than plastic because the test article etched the plastic plates. It was stated the cells did not attach as well to glass culture dishes, which resulted in artificially low relative toxicity at all dose levels. 150 cells were counted per each treatment level. There was no increase in the average net grains per nucleus. No adverse effect. Acceptable. D. Shimer, 7/20/89, J. Gee, 8/2/89.

NEUROTOXICITY, HEN

005 915127, "Examination of DEF (Technical Grade Tributylphosphorotrithioate) from Chemagro Corporation for delayed neurotoxicity" (Mobay No. 27690, 6/8/70); Summary report on prophylactic measures and "neurotoxic esterase" inhibition; two hens were dosed at 200 mg/kg, neurotoxic esterases were measured 17 hours after exposure. Not a guideline type study: not relevant for "possible adverse effects" classification. (A. Apostolou, 5/30/85, and G. Patterson, 6/2/87).

005 915134, "DEF Neurotoxicity Studies on Hens" (Bayer AG, 3/3/76) purity not given, LD_{50} = 306 mg/kg determined in range-finding study; 25/30 hens treated with 300 mg/kg DEF and antidote died prior to day 21, 5 survived through second treatment period; 5 TOCP positive control hens, no negative controls; ataxia and paralysis not reported in DEF treated hens, lymphocytic infiltration reported in sciatic nerve of 2 hens, no neuronal degeneration; NOEL = 300 mg/kg for delayed neuropathy; text report with only brief histopathological findings for surviving hens; UNACCEPTABLE. (A. Apostolou, 5/30/85, and G. Patterson, 6/2/87).

005 915135, "DEF Neurotoxicity Studies on Dermally Treated Hens" (Bayer AG Report No. 6255, 8/5/76) purity not given; dose 5/group once dermally for 24 hour exposure at 0.5, 1.0 or 2.0 ml/kg, observed 28 days; **possible adverse effect**: demyelination, vacuolar distension, axon disintegration; apparent NOEL = 0.5 ml/kg; UNACCEPTABLE, summary report. (A. Apostolou, 5/30/85, G. Patterson, 6/3/87).

EPA one-liner: Minimum. NOEL = 0.5 ml DEF/kg (uncoordinated leg movement minimal to moderate nerve fiber degeneration).

005 915136, "DEF Neurotoxicity Studies on Hens (Inhalation Experiments)" (Bayer AG Report No. 6444, 10/26/76) DEF 94%; Single doses were given to 5/group 5/group at 391, 878 or 1585 mg/m^3 (4 hour exposures). Five 4 hour exposures were given to 10/group at 62, 145 or 256 mg/m^3. Hens were observed for 21 days. Ataxia and paresis were observed, which were delayed in onset and continued to 21-day term of study, had an apparent NOEL = 878 mg/m^3 for single 4 hr exposure and 62 mg/m^3 for five 4 hr exposures; histopathic alterations (axon disintegration and demyelination) in 2/5 hens at single exposure to 1585 mg/m^3, and in 5/10 with 5 exposures to 256 mg/m^3. These findings are considered a **possible adverse effect**. UNACCEPTABLE (duration of study was not consistent with current standards, other major deviations from guidelines), useful information provided. (A. Apostolou, 5/30/85, and G. Patterson, 6/3/87).

EPA one-liner: Minimum. NOEL = 62 mg/m^3 (uncoordinated leg movements).

016 028353, "Study of the Low level Effects of Organophosphorus Esters with Specific Reference to the Delayed Neurotoxic Syndrome" (Duke University Medical Center, Durham, NC, 1/27/78) DEF 95%, single dose via oral gavage to 11 groups of hens, 3 hen/group in dose range from 50 - 1000 mg/kg, observed 3 mo.; apparent NOEL = 50 mg/kg, LEL = 100 mg/kg; ataxia, weakness, but no histological abnormalities observed; originally reviewed by A. Apostolou, 5/29/85 and found acceptable with **possible adverse effects**. However, a rereview finds this study UNACCEPTABLE. Insufficient number of hens/group used, insufficient histopathology data submitted; possible adverse effect could not be confirmed because there was a lack of histopathology data to correlate with clinical observations. (G. Patterson, 9/28/87)

016 017247, Publication of report no. 28353.

016 017246, "Organophosphate Risk Assessment: Field Testing of DEF with the Scaleless Chicken", Bull. Environ. Contam. Toxicol. 24, 921-928 (1980); Univ. of Calif. Davis; this is a field study in which groups of birds were exposed to DEF applications off site, near site, direct application and in lab situations. Not a guideline type study. Cholinesterase inhibition observed. (A. Apostolou, 5/30/85, and G. Patterson, 6/5/87).

016 017245, "Delayed Neurotoxic and Late Acute Effects of S,S,S-Tributylphosphorotrithioate on the Hen: Effect of Route of Administration." Neurotoxicology 1: 425-447(1979) This study compared the "acute", "late effect" and "delayed neurotoxic" effects of DEF by oral and topical administration. Clinical observations included: (1) acute toxicity (typical of ChE inhibition, and responsive to atropine treatment), (2) a "late effect", which begins within 2 days of treatment and which may continue with progressive neurological effects leading to death, all in the absence of neurohistopathological signs, and (3) TOCP-like delayed neurotoxicity with associated microscopic lesions. Neurohistopathological changes comparable to TOCP effects were seen only at 1000 mg/kg (HDT) dermal dose of DEF; oral dosing of DEF did not lead to neurohistopathological changes. Investigators concluded that the "late effect" was caused by n-butyl mercaptan (the major metabolic product formed in the digestive tract), and that delayed neurotoxicity" was caused by DEF. Only in dermal exposure studies are subjects exposed to appreciable amounts of unmetabolized DEF. UNACCEPTABLE, but useful data. (A. Apostolou, 5/30/85, and G. Patterson, 6/5/87).

019 036679, "Organophosphorus and Other Inhibitors of Brain 'Neurotoxic Esterase' and the Development of Delayed Neurotoxicity in Hens", (Medical Research Council Tox. Unit, UK, published article, 1970). DEF, >99% purity, 220 and 1100 mg/kg apparently given as single sc dose, "Neurotoxic esterase" inhibition of 35 and 77% respectively for the two doses. UNACCEPTABLE (not a guideline study). (C. Aldous, 4/16/86).

019 036680, "Effects of a Dermal Dose of S,S,S,-Tri-N-butyl phosphorotrithioate on Brain Acetylcholinesterase Acid Phosphatase, and 2', 2'-cyclic nucleotide-3'phosphohydrolase and Plasma Butrylcholinesterase in Hens" (Duke Univ. Med. Center Dept. of Pharmacol., publ. article, 1983). DEF (95%, presumed tech.), 100, 250, 500, 750, or 1000 mg/kg DEF given with a single dermal administration. NOEL was less than or equal to 100 mg/kg (ataxia at 250 mg/kg). Acute cholinesterase inhibition clinical signs at 500+ mg/kg. Dose-related classical TOCP-type histopathological picture reported (data not shown). UNACCEPTABLE. (C. Aldous, 4/16/86).

NEUROTOXICITY, SUBCHRONIC, HEN

RECOMMENDATIONS:

The neurotoxicity study submitted by Mobay Corp. (Record # 89360) was performed according to EPA guidelines and was found to be acceptable to fill the data gap for subchronic delayed neurotoxicity. The registrant performed the study using the dermal route of exposure, as recommended by Patterson, 11/19/87. Kellner, 6/24/91.

**** 272-051 089360** Sheets, L. "Subchronic Delayed Neurotoxicity Study with Technical Grade Tribufos (DEF®) in Hens" (Mobay Corporation, Corporate Toxicology Department, Report 100006, 2/26/91). Tribufos, Batch # 85R-26-39, 97.7% pure at 0 (control), 2.6, 11 and 42 mg/kg/day administered daily (five days/week) by dermal application to the comb of adult White Leghorn hens (12/dosage) for a total of 65 applications in 13 weeks; twelve hens received 18 mg/kg/day of TOCP (positive control). Clinical signs of decreased motor activity and ataxia consistent with delayed neurotoxicity (ataxia scores ranging from 1 to 4 on a scale of 5) were observed at 42 mg tribufos. Whole-blood cholinesterase was reduced by 50-60% throughout treatment period at all dose levels. **Possible adverse effects**, ataxia at 42 mg/kg and axonal degeneration in spinal cord at 11 and 42 mg/kg doses. NOEL = 2.6 mg/kg/day for histopathologic lesions. ACCEPTABLE (Kellner and Gee, 4/30/91).

019 036676 and 036677, "Potentiation and Neurotoxicity Induced by Certain Organophosphates in Mice and Chickens" (Univ. Wisconsin Dept. Entomology, 1963) DEF, purity not given; mice were given 100 mg/kg (followed by secondary dose of malathion), Hens were given 100 mg/kg/day ip for 10 days w/o atropine. No NOELs: Mouse treatment enhanced lethality of malathion. Hen treatment caused ataxia after 10 days. (No data provided). UNACCEPTABLE (Journal article surveying many pesticides). (C. Aldous, 4/15/86).

019 036678, "Neurological Disruption Produced in Hens by Two Organophosphate Esters: Intraperitoneal and Oral Administration (DEF - S,S,S-Tributylphosphorotrithioate)", (FDA Div. Pharmacol., (Publ. in Brit. J. Pharmacol. 23:295, 1964). DEF (grade and purity not given), 50 and 100 mg/kg/day given ip and orally in corn oil solutions, also 150 ppm given orally in corn oil. No NOEL established with either administration mode. **Possible Adverse Effect**: Substantial microscopic pathology with either administration mode. Appreciable clinical signs with ip dosing only. UNACCEPTABLE. (C. Aldous, 4/16/86).

005 915129, "DEF Neurotoxic Effects on Poultry (Subacute Inhalation Study)" (Bayer AG Report No. 7614, 6/15/78). DEF 95%, dose given with statically nebulized paint spray gun for 1/2 min. every 30 minutes. Dose 15 times for 6 hours at 0, 8, 21 or 84 mg/m^3. Apparent delayed neuropathic NOEL for 15 X 6 hour exposures = 21 mg/mg^3; **Possible adverse effect**: sciatic nerve axonal degeneration and myelin sheath vacuolation in 1/5 hens. Not a guideline type study. (A. Apostolou, 5/30/85, and G. Patterson, 6/4/87).

005 915137, "DEF Studies of Subacute Dermal Neurotoxic Effects on Poultry" (Bayer AG Report No. 8031). DEF 95%; DEF administered dermally 15 times for 6 hour exposures to groups of 8 hens at 0, 0.01, 0.03, 0.1, 0.3 or 1.0 ml/kg then observed for 28 days. Mortalities in 2 highest dose levels; **Possible Adverse Effect**: delayed neurotoxic effects reported, apparent NOEL = 0.03 ml/kg. Not a guideline type study. (A. Apostolou, 5/30/85, and G. Patterson, 6/4/87).

005 915145, "Report on Demyelination Studies on Hens"; (Harris Laboratories, Inc. 4/17/65, 7/22/65, study repeat). DEF, purity not stated; 6 hens/group, dose in feed for 30 days at 0, 100, 250, or 500 ppm, then observed for 30 days; Summary report, Not a guideline type study, no

analysis of dose, no abnormal symptoms observed and no evidence of demyelination reported, no positive control, no data. (A. Apostolou, 5/30/85, and G. Patterson, 6/2/87).

005 915128, "DEF Neurotoxicity Studies on Hens (Thirty-Day Feeding Experiments)", (Bayer AG Report No. 6941, 8-15-77). DEF 95%, 10/group at 0, 25, 50, 100, 200, or 400 ppm in feed for 30 days followed by 30 day observation; dose-related perivascular CNS and PNS inflammation, no symptoms of neurotoxicity reported; Not a guideline type study - no analysis of treated feed. (A. Apostolou, 5/30/85, and G. Patterson, 6/8/87).

016 017244, "Effects of Chronic Oral and Dermal Administration of Pesticides on Laying Hens, Including Delayed Neuropathy 1. Organophosphorus Pesticides", (Univ. of Illinois, Urbana, IL, 9-82 (report of 7 op's and 2 thiocarbamates). DEF 92% purity, administered orally by gelatin capsules (10% or 20% w/w in corn oil or dermally to ventral wing surface (20 % E.C. in xylene with 2% Triton X-100) for 91 to 101 days. **Possible adverse effect indicated**: 3/3 hens treated orally with 5.0 to 5.9 mg/kg for 91 days or 3.0 to 3.7 mg/kg for 97 days showed no clinical symptoms of delayed neurotoxicity; 2/3 hens treated with 11.7 to 15.6 mg/kg dermally for 91 days and 3/3 hens treated with 5.9 to 7.8 mg/kg dermally for 101 days displayed **clinical symptoms of delayed neurotoxicity**. UNACCEPTABLE, insufficient number of animals, submitted data (except for clinical observations) in summary form, no histopathology data. (A. Apostolou 5/30/85, and G.Patterson, 6/10/87).

016 017243, "Delayed Neurotoxic, Late Acute and Cholinergic Effects of S,S,S-Tributyl phosphorotrithioate (DEF) Subchronic (90 days) Administration in Hens", (Toxicology, 14 (1979) 229-243, Duke Univ. Medical Center). DEF (95% purity), given daily for 90 days via oral route in gelatin capsules to 5 hens/group at 0, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, 40.0 or 80.0 mg/kg and 5 hens/group treated with TOCP or parathion as positive and negative controls, respectively. Three hens/group were treated dermally daily for 90 days with 20 or 40 mg/kg DEF; 20 mg/kg TOCP (positive control). All hens surviving the treatment period were examined 30 days after dosing period prior to sacrifice. Onset and severity of ataxia was dose-related for both oral and dermal applications. Definitive histopathic evidence of neural degeneration was limited to 1 hen of the 80 mg/kg/day oral group and to 3/3 hens of the 40 mg/kg/day dermal treatment group. The oral NOEL for "clinical signs of delayed neurotoxicity" = 0.1 mg/kg/day: the topically-treated hens all demonstrated gross ataxia at the lowest dose of 20 mg/kg/day. UNACCEPTABLE, useful data - need 10 hens/group per guidelines, body weight data insufficiently summarized, more detailed histopathology description necessary. (A. Apostolou, 5/30/85, and G. Patterson, 6/1/87).

016 028352, Study of the Low Level Effects of Organophosphorus Esters with Specific Reference to the Delayed Neurotoxic Syndrome", (Duke Univ. Medical Center Durham, NC 1/27/78). DEF, 95% purity, hens dosed by gavage for 90 days 5/dose at levels of 0, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0 mg/kg. Apparent NOEL = 0.1 mg/kg/day; ataxia (all hens in 0.5 to 20 mg/kg group), paralysis, sciatic nerve degeneration 1/5 at 20 mg/day. UNACCEPTABLE too few hens per dose group, age not stated, no analysis of dose, individual histopathology not included. (A. Apostolou, 5/29/85, and G. Patterson, 6/8/87).

025 051534/5, "Subchronic Delayed Neurotoxicity Hens", (Bayer, W. Germany 10/76). DEF technical (95% purity), administered at 100 ppm (6.03 mg/kg/day), 200 ppm (10.9 mg/kg/day) or 400 ppm 18.5 mg/kg/day) in feed for 30 days to 10 hens/group. No clinical symptoms of neurotoxicity observed; blood ChE activity inhibited in all treated groups; **Possible Adverse Effect**: histopathic evidence of neural degeneration in two highest dose groups; no mortalities; neurohistopathic NOEL = 6.03 mg/kg/day; Not a guideline study. (G. Patterson, 3/30/87).

NEUROTOXICITY, RATS

** 272 – 110, 112, 113 177089, 182336, 182337 "An acute oral neurotoxicity screening study with technical grade Tribufos (DEF®) in Wistar rats" (L. P. Sheets and R. G. Gilmore, Bayer Corporation, Stilwell, Kansas, Report Number 109802, 7/31/2000, 109803, 6/19/00 and 109806, 9/16/99) Male and female Wistar rats were given a single dose of tribufos (DEF®) technical (98.5%) by oral gavage in corn oil at nominal doses of 0, 2, 20 and 100 mg/kg. There were 12 animals/sex/group in the main study and 6/sex/group for cholinesterase determination approximately 6 hours after dosing (the time of maximum cholinergic effect). Plasma, erythrocyte and whole brain cholinesterase activity were measured both pretreatment and at 6 hours on day of treatment. Functional observational battery (FOB) and motor testing were performed. Six randomly selected males and females per group were perfused for tissue collection with tissues from the control and high dose groups examined for micropathology. Brain weights were recorded. Results: Acute cholinergic signs reported on day of treatment at the high dose included decreased activity, clear lacrimation, lower body temperature, various stains, posture (lying flattened), decreased rears in the open field and decreased reaction to an auditory stimulus. At 20 mg/kg, animals showed a decreased response to auditory stimulus and decreased arousal in the open field. Forelimb and hindlimb grip and landing foot splay were not affected. Motor and locomotor activities were significantly decreased at the high dose in both sexes and in males at 20 mg/kg. Effects noted in the FOB and activity measurements had returned to normal by day 7 in the mid dose group but were marginally lower at 100 mg/kg. At termination, the high dose males had returned to normal activity but the high dose females still had lower activity (30%) relative to controls. Plasma and erythrocyte cholinesterase activities were significantly inhibited at 20 and 100 mg/kg in both sexes at approximately 6 hours after dosing. Brain cholinesterase was not affected in males but was 16% lower than controls in females at 100 mg/kg (statistically significant, $p < 0.05$). There was no affect on brain weight or micropathology. No adverse effect. Originally reviewed as unacceptable but upgradeable with submission of appropriate positive control data (excluding carbaryl). (Gee, 9/18/2000) Documents 272-112 and 272-113 contain the appropriate positive control data to upgrade the study to ACCEPTABLE status. (Gee, 5/15/02)

**** 272 – 111, 112, 113 180406, 182336, 182337** "A subchronic neurotoxicity screening study with technical grade tribufos (DEF®) in Wistar rats." (Sheets, L. P. and R. G. Gilmore with pathology report by B. P. Stuart, Bayer Corporation, Stilwell, KS, Study number 99-N72-BB, Report number 110242 and 109070 (diet analysis), 3/26/01). DEF was fed in the diet for 13 weeks to Wistar rats [Crl:WI[G1x/BRL/Han]IGS BR], 18/sex/dose, at nominal levels of 0, 2, 40 and 500 ppm, equivalent to doses of 0, 0.14, 2.89 and 36.8 mg/kg/day for males and 0, 0.17, 3.54 and 42.6 mg/kg/day for females. Twelve per sex per dose group were used for neurobehavioral study with the remaining 6 used for cholinesterase determination pretest and weeks 4 and 13 (plasma and RBC plus brain at termination). Of the twelve, six/sex/group were perfused for micropathology with an emphasis on brain and nerve tissues, including the eye and optic nerve. Effects noted at 500 ppm included lower body weights and food consumption, decreased motor and locomotor activity, and retinal atrophy (degeneration), usually bilateral, with incidences of 5/6 in males and 6/6 in females compared with 0/6 in both control groups. In addition, 1/6 males had degeneration of the gasserian ganglion or the sciatic nerve. No findings were noted in the eyes of the 40 ppm groups. The NOEL for cholinesterase inhibition was 2 ppm with significant inhibition of plasma and RBC cholinesterase at 40 and 500 ppm, weeks 4 and 13. Brain cholinesterase activity was significantly depressed at week 13 in males (-63%) and females (-74%) at 500 ppm. Brain activity in females was –8% at 40 ppm and was statistically significant. Activity in males was comparable to controls. The systemic NOEL was 40 ppm (lower body weight, decreased motor and locomotor activity and retinal atrophy). **Possible adverse effect: retinal atrophy.**

Initially reviewed as unacceptable, possibly upgradeable (possibly upgradeable with submission of suitable positive control data.) (Gee, 5/16/01). Documents 112 and 113, records 182336 and 182337, contain appropriate control data and upgrade the study to ACCEPTABLE status. (Gee, 5/15/02)

** 272 - 114 182690 Lake, S. G. "A Developmental Neurotoxicity Screening Study with Technical Grade Tribufos (DEF®) in Wistar Rats". (Bayer Corporation, Stilwell, Kansas, Study numbers 00-D72-AG and 00-D72-AS, Report no. 110523, July 27, 2001.) Technical Grade Tribufos (DEF®) was admixed with the feed at nominal concentrations of 0, 4, 40 and 200 ppm from gestation day 0 through lactation day 21 and fed to 15 and 30 mated Wistar Crl:W(HAN)BR females rats/dose in satellite and main study groups, respectively. Maternal body weight was reduced 8-12% during lactation at 200 ppm. Slight tremors were noted in five dams at the high dose on the day of parturition only. Cholinesterase inhibition in blood, plasma, and brain was measured on day 21 of lactation, the only day assayed for dams, with significant reduction in activity at 40 and 200 ppm. Maternal ChE NOEL = 4 ppm. Overall maternal NOEL = 40 ppm. High dose pup bodyweights and food consumption were significantly lower. There was also a slight delay in surface righting and preputial separation but not in vaginal opening. The auditory startle response on PND 22 was reduced in peak amplitude at the high dose. Plasma cholinesterase activity was significantly reduced on day 21 in male and female pups (but not at day 11) at 200 ppm. Brain cholinesterase in female pups was statistically significantly inhibited on day 11 (-6%) but not in male pups or in either sex on day 21. There was a transient decrease in brain measurement (cerebrum length) at day 11 but day 21 values were comparable to controls. The only persistent effect was lower body weight at the high dose. Pup ChE NOEL = 40 ppm. Overall pup NOEL = 40 ppm. ACCEPTABLE. (Kishiyama and Gee, 10/3/01)

272 - 112 182336 "A motor activity historical control and method validation study using triadimefon and chlorpromazine in Wistar rats." (Sheets, L. P. and G. L. Armintrout, Bayer Corp. Agriculture Division, Report no. 109803, 6/19/2000) This report presented positive control data cited in the above study in record no. 182690. Young adult male Wistar rats in groups of 12 were treated with 0 (untreated), 0 (PEG 400), chlorpromazine in saline (2 mg/kg by ip injection) and triadimefon in PEG 400 (177 mg/kg by oral gavage) in a single dose. Triadimefon increases activity and chlorpromazine decreases motor activity. Animals were tested in figure-eight mazes over 90 minutes in 10-minute intervals for motor activity, locomotor activity and habituation. The results were as expected with triadimefon increasing activity approximately three-fold and chlorpromazine decreasing activity by approximately 40%. No worksheet. (Gee, 10/5/01)

272 - 113 182337 "Verification of personnel training to perform a functional observational battery with rats." (Sheets, L. P. and R. G. Gilmore, Bayer Corp., Agriculture Division, Report no. 109806, 9/16/99) This report presented the results of a periodic demonstration of inter-observer reliability for the neurotoxicity screening battery. Young-adult male Fischer 344 rats were used with 10/technician in the initial untreated group. Following the initial phase, 6/dose level were given carbaryl ip at doses of 0, 17 or 32 mg/kg. Five observers recorded the results concurrently, without knowledge of the treatment group. One technician served as the lead. The FOB was conducted once, shortly after treatment. Carbaryl caused dose-related neurological effects. Four of the five technicians had good overall agreement. One technician, however, was judged as needing more training. No worksheet. (Gee, 10/5/01).

SUBACUTE INHALATION STUDY

272-001 915133 Thyssen, J. "DEF Subacute Inhalation Study on Rats" (Bayer AG, Institut Fur Toxikologie, Wuppertal-Elberfeld, Report# 7649, 6/22/78). Tribufos technical, formula 605042, batch 5030063, purity 95%, was administered to 10 Wistar II rats/sex/dose at concentrations of 0, 2, 7 and 32 mg/m^3 of air for 3 weeks (6 hours/day, 5 days/week). High-dose rats had slight behavioral abnormalities, including lethargy and decreased preening; slight lung inflammation and increased adrenal weights were seen in this group at necropsy. Marked inhibition of plasma ChE activity was recorded at 7 and 32 mg/m^3; slight inhibition of erythrocyte and brain ChE (about 75% of control activity remaining) was seen at the high dose level. NOEL = mg/m^3 (0.84 mg/kg/day, based on brain ChE inhibition and behavioral abnormalities at 32 mg/m^3). Not a guideline study; no worksheet. Lewis, C. and Kellner, T., 1/24/92.